

Khat Extraction Procedure:

1. Separate leaves from stems
2. Soak 20-25 g in 0.1 N H₂SO₄, sonicate 15 -20 minutes
3. Vacuum filter acidic solution through fiberglass wool
4. Extract acidic layer with CHCl₃ three times
5. Discard CHCl₃ layers
6. Basify acid layer with 5% Na₂CO₃ followed by extracting 3 times with MeCl₂.
7. Filter MeCl₂ extracts over MgSO₄
8. Solvent evaporation {air dried or steam bath (with caution)}

LC-MS Analysis:

Sample Preparation:

- The methylene chloride extract evaporated to dryness and then reconstituted in few drops of LC-MS grade Methanol and 2 ml of 10 mM Ammonium Formate, pH 3.7

Method Parameters:

- *Instrumentation:* Agilent Technologies 1100 Series high performance liquid chromatograph (HPLC) equipped with a quaternary pump, vacuum degasser, autosampler, thermostatted column compartment, and a diode array detector coupled to an Agilent Technologies mass analyzer Model SL 6100 single quadrupole equipped with an electrospray ionization interface
- *Column:* Synergi Phenomenex Polar-RP (150 x 3.00 mm, 4 μ m 80 \AA)
- *LC conditions:* Solvent (A) 93% 10mM ammonium formate pH 3.7 : Solvent (B) 7% ACN, delivered isocratically at 0.500 ml/min; Column Temp at 40° C
- *UV DAD:* Signal#1: 250 nm, Signal#2: 210nm, 10nm
- *MSD conditions:* ESI positive polarity scan; step size 0.15 amu; capillary voltage 3000V, N2 drying gas 13 l/min at 350° C, nebulizer at 30 psi; variable fragmentor ramp: MSD1 Signal (Cathinone) m/z150(100V), m/z132(150V), m/z117(200V), m/z50(300V); MSD2 Signal (Cathine), m/z152 (80V); m/z134 (140V); m/z91 (240V)

Agilent's 1200 Series Binary Pump Model S/L Rapid Resolution HPLC coupled to a 6130A Single Quadrupole MSD can also be used to achieve similar results in shorter amount of time. Conditions remain the same except for the column; Synergi Phenomenex Polar-RP column, 50 x 2.00 mm 2.5 μ m 80 \AA , should be used instead.